

Data Evaluation Report on the acute toxicity of Novaluron on the Freshwater Alga *Selenastrum capricornutum*

PMRA Submission #: {.....}

EPA MRID #: 45638221

Data Requirement: PMRA DATA CODE {.....}
EPA DP Barcode D285479
OECD Data Point {.....}
EPA MRID 45638221
EPA Guideline 122-2

Test material: "RIMON" Technical Purity: 99.3%
Common name: Novaluron
Chemical name: IUPAC: 1,(3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/03

QC Reviewer: Dana Worcester
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/03

Primary Reviewer: Bill Evans
{EPA/OECD/PMRA}

Date: {.....}

11/17/03

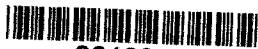
Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA}

Date: {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 124002

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Jenkins, C.A. 1998. "RIMON" Technical: Algal Growth Inhibition Assay. Unpublished study performed by Huntingdon Life Sciences Ltd, Suffolk, England. Laboratory Project Identification No. MAK/449. Study submitted by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel. Sponsor Study No. R-9842. Experimental start date September 1, 1997 and experimental termination date September 5, 1997. The final report issued March 30, 1998.



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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Novaluron under static conditions. The single nominal concentration was 10 mg/L. The mean measured concentration was 9.68 mg/L, this treatment group was compared to a dilution water and solvent control. There were no effects on cell density, growth rate, or area under the growth curve (biomass) in the 9.68 mg/L treatment group compared to the solvent control. As a result, the NOEC was 9.68 mg/L and the EC₅₀ was >9.68 mg/L.

The study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §122-2 for an aquatic nonvascular Tier 1 plant study with *Selenastrum capricornutum*. However, since the required light intensity measurements of 4-5 Klux ($\pm 15\%$) were not used for the test, this study must be classified as Supplemental.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell Density:

NOEC: 9.68 mg/L
EC₅₀: >9.68 mg/L 95% C.I.: N/A

Growth rate:

NOEC: 9.68 mg/L
EC₅₀: >9.68 mg/L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):

NOEC: 9.68 mg/L
EC₅₀: >9.68 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD Procedure 201 (1984); EC Directive 92/69/EEC, Part C3 (1992); and U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget plants, §122-2h. The following deviations from U.S. EPA Guideline 122-2 are noted:

1. The length of the acclimation period and age of the inoculum were not specified.
2. The light intensity where at the position of the culture flasks was estimated at 8.250 Klux; however, measurements with a Radiospeares luxmeter showed 2.0-2.1 Klux when a single tube was illuminated. EPA requires that the light intensity be $4-5 \pm 15\%$ Klux.
3. The material of the test vessels was not reported.
4. The dilution water was dechlorinated. The pH of the dilution water was not reported.
5. The agitation rate of 175 rpm was greater than recommended (100 rpm).

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material "Rimon" Technical (Novaluron)

Description: Fine, purple-tinted powder

Lot No./Batch No. : 970211/4

Purity: 99.3%

Stability of Compound

Under Test Conditions: The measured concentrations of Novaluron was 109% of nominal at hour 0 and 85% of nominal at hour 96. The 96 hour measured concentration was 78% of 0 hour measured concentration (Table 1, p. 19).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Water Solubility: 3 µg/L

Storage conditions of test chemicals: The test substance was stored under ambient conditions protected from light.

2. Test organism:

Name: *Selenastrum capricornutum*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S.

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costatum, *A. flos-aquae*, *S. capricornutum*, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: *S. capricornutum*, *S. subspicatus*, and *C. vulgaris*. If other species are used, the strain should be reported

Strain: CCAP number 278/4

Source: Originally from Culture Collection of Algae and Protozoa (CCAP), Freshwater Biological Association, Ferry House, Ambleside, Cumbria. In-house laboratory cultures.

Age of inoculum: Several days old (age not specified).

Method of cultivation: Sterile OECD medium (Appendix 2, p. 23)

B. STUDY DESIGN:

a) Range-finding Study: A range-finding study with Novaluron was conducted in order to estimate the nominal test concentration for the definitive study. Triplicate cultures of algae were exposed for 96 hours at nominal concentrations of 0.1, 0.1, 1, and 10 mg/L. Based on cell counts, the EC_{50} was greater than the measured 7.32 mg/L treatment group and the NOEC was ≥ 7.32 mg/L.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: culturing media and conditions: (same as test or not)	Several days Sterile OECD medium (Appendix 2, p. 23); same as test.	The acclimation period was not specified.
health: (any toxicity observed)	Not reported	EPA recommends two week acclimation period. OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Incubator	

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Parameter	Details	Remarks
		Criteria
Duration of the test	96 hours	<p><i>EPA requires: 96 - 120 hours</i></p> <p><i>OECD: 72 hours</i></p>
Test vessel material: (glass/polystyrene) size: fill volume:	Sterilized conical flasks with non-absorbent cotton wool plugs 250 mL 50 mL	<p>The material of the test vessels was not reported.</p> <p><i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i></p>
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	OECD mineral salts medium (see Appendix 2, p. 23) 7.5 7.2-7.5 Na ₂ EDTA·2H ₂ O (0.1 mg/L) NaHCO ₃ (50.0 mg/L) N/A	<p><i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i></p> <p><i>EPA recommends 20X-AAP medium.</i></p>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Laboratory tap water Filtered, dechlorinated Not reported N/A Reverse osmosis Not reported Not reported Not reported Not reported Not reported	<p>The dilution water was dechlorinated. The pH of the dilution water was not reported.</p> <p><i>EPA pH: <u>Skeletonema costatum</u> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.</i></p> <p><i>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.</i></p>

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Parameter	Details	Remarks
		Criteria
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 175 rpm	<p>The agitation rate of 175 rpm was greater than recommended (100 rpm).</p> <p><i>EPA recommends agitation only for <u>Selenastrum</u> at 100 cycles per min and <u>Skeletonema</u> at ~60 cycles per min. Aeration is not recommended.</i></p>
Initial cells density	Approximately 10,000 cells/mL	<p><i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <u>Selenastrum capricornutum</u>, cell counts on day 2 are not required.</i></p> <p><i>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <u>S. capricornutum</u> and <u>S. subspicatus</u>. When other species are used the biomass should be comparable.</i></p>
Number of replicates control: solvent control: treated ones:	6 6 6	<p>Four extra replicates in each control and treatment group were used for environmental measurements and chemical analysis (not incubated).</p> <p><i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <u>Navicula</u> sp. tests should be conducted with four replicate.</i></p> <p><i>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.</i></p>

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Parameter	Details	Remarks
		Criteria
Test concentrations nominal:	10 mg/L	The mean measured concentration was determined from 0 and 96 hour mean recoveries (Table 1, p. 19).
measured:	9.68 mg/L	<i>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</i> <i>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.</i>
Solvent (type, percentage, if used)	Acetone (0.1 mL/L)	
Method and interval of analytical verification	HPLC; 0 and 96 hours	
Test conditions temperature: photoperiod: light intensity and quality:	23.8-24.6°C Continuous 8250 lux, cool-white fluorescent lighting	<i>EPA temperature: <u>Skeletonema</u>: 20°C, Others: 24-25°C; EPA photoperiod: <u>S. costatum</u> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <u>Anabaena</u>: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)</i> <i>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</i>
Reference chemical {if used} name: concentrations:	Potassium dichromate Not reported	72 hour biomass EC ₅₀ was 0.47 mg/L (95% confidence limits 0.39 and 0.57 mg/L).
Other parameters, if any	None	

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2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell count, growth rate, and area under the growth curve	<i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</i>
Measurement technique for cell density and other end points	Haemocytometer	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i>
Observation intervals	Every 24 hours	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water control group cell density at test termination was 105X greater than the dilution water control group cell density at test initiation.	<i>EPA requires control cell count at termination to be $\geq 2X$ initial count or by a factor of at least 16 during the test.</i> <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

There were no effects on cell density, growth rate, or area under the growth curve (biomass) in the mean measured 9.68 mg/L treatment group compared to the solvent control.

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Table 3: Effect of Novaluron on freshwater alga (*Selenastrum capricornutum*)

Treatment mean measured and nominal concentrations ^a (mg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition ^b
Dilution water control	~10,000	74,000	1,050,000	--
Solvent control	~10,000	79,000	1,110,000	--
9.68 (10)	~10,000	74,800	1,010,000	9.0
Reference chemical (if used)	N/A	N/A	N/A	N/A

^a The nominal test concentration is presented in parentheses.

^b The % inhibition was reviewer-calculated from mean cell density data compared to the solvent control.

Table 4: Effect of Novaluron on the freshwater alga *Selenastrum capricornutum*

Mean Measured and Nominal Treatment Concentrations ^a (mg/L)	Initial cell density (cells/mL)	Mean Growth Rate per day (x 10 ⁻²)	% inhibition (Mean Growth Rate per day) ^b	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve) ^b
Dilution water control	~10,000	4.834	--	44,290,000	--
Solvent control	~10,000	4.901	--	37,950,000	--
9.68 (10)	~10,000	4.805	2	34,100,000	10
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

^a The nominal test concentration is presented in parentheses.

^b The % inhibition is compared to the solvent control.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC ₀₅ (mg/L)	9.68	9.68	9.68
EC ₅₀ (mg/L)	>9.68	>9.68	>9.68
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	Not reported	Not reported	Not reported
other (IC ₂₅ /EC ₂₅)	Not reported	Not reported	Not reported

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Statistical Endpoint	Biomass	Growth rate	Cell density
Reference chemical, if used NOAEC IC ₅₀ /EC ₅₀	N/A	N/A	N/A

N/A = Not applicable.

B. REPORTED STATISTICS:

Statistical Method: The formulas used to calculate the area under the growth curve and growth rates are presented on p. 13. The Dunnett's multiple comparison test (multiple t-test) was used to compare the treatment group to the solvent control. The EC₅₀ for growth rates and biomass were estimated based on percent inhibitions. The reported statistics were based on the measured test concentration.

Cell Density:

NOEC: Not reported

EC₅₀: Not reported

Growth rate:

NOEC: 9.68 mg/L

EC₅₀: >9.68 mg/L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):

NOEC: 9.68 mg/L

EC₅₀: >9.68 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Data for cell density, growth rate, and biomass were analyzed. For all endpoints, the negative control data were first compared to the solvent control data using a Student's t-test. No differences were found between these groups for any endpoint. As a result, the pooled control was compared to the treatment group to determine the NOEC. Reductions did not exceed 50% so the EC₅₀ was determined visually.

Cell Density:

NOEC: 9.68 mg/L

EC₅₀: >9.68 mg/L 95% C.I.: N/A

Growth rate:

NOEC: 9.68 mg/L

EC₅₀: >9.68 mg/L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):

NOEC: 9.68 mg/L

EC₅₀: >9.68 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

The light intensity was estimated at 8.25 Klux. The required intensity for EPA studies is 4-5 Klux ($\pm 15\%$).

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's.

One solvent control replicate had an anomalously low cell number value. This replicate was not used in the calculation of mean values.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular Tier 1 plant study with *Selenastrum capricornutum* [§122-2]. However, since the required light intensity measurements of 4-5 Klux ($\pm 15\%$) were not used for the test, this study must be classified as Supplemental. There were no effects on cell density, growth rate, or area under the growth curve (biomass) in the 9.68 mg/L treatment group, compared to the pooled control. The EC_{50} was >9.68 mg/L and the NOEC was 9.68 mg/L.

Cell Density:

NOEC: 9.68 mg/L

EC_{50} : >9.68 mg/L 95% C.I.: N/A

Growth rate:

NOEC: 9.68 mg/L

EC_{50} : >9.68 mg/L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):

NOEC: 9.68 mg/L

EC_{50} : >9.68 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

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III. REFERENCES:

US EPA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 122 Tier 1 of Non target area testing, 122-30 (e) *Selenastrum capricornutum*: growth conditions, October 1982.

OECD Guidelines for Testing of Chemicals, Procedure 201. "Alga, Growth Inhibition Test", adopted 7 June, 1984.

Official Journal of the European Communities. L383A. Part C: Methods for Determination of Ecotoxicity: C.3. Algal Inhibition Test. Vol 35, 29 December 1992. ISSN 0378-6978.

Dunnett, C.W. (1955) A multiple comparison procedure for comparing several treatments with a control. Journal of American Statistical Association, 50, 1096-1121.

Dunnett, C.W. (1964) New tables for multiple comparisons with a control. Biometrics, 20, 482-491.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell density

Standard Two-Sample t-Test

data: neg control: V1 in DS2 , and solvent control: V2 in DS2
t = -0.7275, df = 9, p-value = 0.4854
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-23.49370 12.06037
sample estimates:
mean of neg control: 104.8833
mean of solvent control: 110.6

Standard Two-Sample t-Test

data: pooled control: V1 in DS2 , and 9.68 mg/L: V3 in DS2
t = 1.0763, df = 15, p-value = 0.2988
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-6.239877 18.970180
sample estimates:
mean of pooled control: 107.4818
mean of 9.68 mg/L: 101.1167

Growth rate

Standard Two-Sample t-Test

data: neg control: V1 in DS3 , and solvent control: V2 in DS3
t = -0.7619, df = 9, p-value = 0.4656
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-0.2694908 0.1336908
sample estimates:
mean of neg control: 4.8335
mean of solvent control: 4.9014

Standard Two-Sample t-Test

data: pooled control: V1 in DS3 , and 9.68 mg/L: V3 in DS3
t = 0.8993, df = 15, p-value = 0.3827
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-0.08133965 0.20006692
sample estimates:
mean of pooled control: 4.864364
mean of 9.68 mg/L: 4.805

Biomass

Standard Two-Sample t-Test

data: neg control: V1 in DS4 , and solvent control: V2 in DS4
t = 0.6443, df = 9, p-value = 0.5354
alternative hypothesis: true difference in means is not equal to 0

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95 percent confidence interval:

-754.0681 1354.7347

sample estimates:

mean of neg control: 4095.333

mean of solvent control: 3795

Standard Two-Sample t-Test

data: pooled control: V1 in DS4 , and 9.68 mg/L: V3 in DS4

t = 1.742, df = 15, p-value = 0.102

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

-122.6355 1219.6052

sample estimates:

mean of pooled control: 3958.818

mean of 9.68 mg/L: 3410.333